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FORMULATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF TOPICAL HERBAL DRUG DELIVERY SYSTEM FOR THE TREATMENT OF ACNE VULGARIS BY USING ALCOHOLIC EXTRACTS

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ABSTRACT
A Novel drug delivery system aim to improve patient compliance, and dispersible are no exception. The dosage forms available for the delivery of topical agents include ointments, pastes, creams, lotions, gels, and powders. Creams are often preferred over the other topical preparations because less irritating and easier to apply. The cooling effect due to evaporation of water gives soothing effect at the inflamed area. The present investigation concerns the developments of formulation and evaluation of efficacious and safe topical herbal drug delivery system for the treatment of Acne Vulgaris by using ethanolic extracts of *Azadirachta indica*, *Amba Haldi* and *Curcuma Longa*. Topical herbal formulation of the plant extract was evaluated for physical parameters, viscosity, Skin Irritation, Spreadibility and antimicrobial activity. The formulations were found to be better in all aspects. Thus it can be used as an alternative for the treatments and drug delivery systems.

KEYWORDS: Topical Herbal Drug Delivery System, *Azadirachta indica*, *Amba Haldi*, *Curcuma Longa*, Acne Vulgaris.

INTRODUCTION
Ayurveda describes acne vulgaris as 'Taraunya Pitika' (during adolescence, eruptions or pimples occur on the face) and 'Mukhadusika' (one that spoils, vitiates or disfigures the face). Currently, available treatment for acne is mostly based on antibiotics and retinoids. The uses of antibiotics have a lot of limitations due to development of resistance by bacteria. Retinoids are highly teratogenic. Therefore, an alternative treatment for acne must be studied and developed. This creates a great interest towards the possible effect of natural substances on anti-inflammatory action in acne lesions. Natural substances that are obtained from the medicinal plant, having antibacterial and anti-inflammatory activity, are commonly employed for the treatment of acne. Herbal drugs are effective against a variety of Gram-positive and Gram-negative bacteria. Sunder Vati, which is an ayurvedic formulation, was found to be orally effective and well tolerated for the treatment of acne vulgaris. Purin tablets and klarina cream formulations, which contain many herbal extracts and have negligible adverse effects compared with modern medicine, are commonly indicated for moderate and severe forms of acne. There are certain herbal extracts, such as *A. dahurica*, *R. coptidis* and *Psidium quajava*, which are more effective than antibiotics and retinoids. The efficacy of these herbal agents in acne treatment is not only based on antimicrobial activity but on their possessed antioxidant and anti-inflammatory properties by which they inhibit neutrophil migration and generation of ROS. Herbal extracts or oil may be used as monotherapy or in combination therapy. The concerned side effects of herbal drugs are much less compared with modern drugs. Thus, natural substances, which are obtained from the medicinal plant, having antibacterial and anti-inflammatory activity, are commonly employed for the treatment of acne.[1,2]

Present aim of the work is Development and evaluation of efficacious and safe topical herbal drug delivery system for the treatment of Acne Vulgaris by using modern pharmaceutical techniques for better efficacy, stability and sensory, because topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations.

MATERIALS AND METHODS

White soft paraffin, Unicorn petroleum (Gift sample), Cetostearyl alcohol, Savita chemicals(Purchased), Cetyl alcohol, Savita chemicals(Purchased), Isopropyl Myristate, Savita chemicals (Purchased), Mineral Oil, Savita chemicals (Purchased), Sorbitan Monostearate, Fine Organics (Gift sample), Polysorbate 60, Fine Organics (Gift sample), Methyl Paraben, Godrej ind. Ltd. (Gift sample), Propyl Paraben, Godrej ind. Ltd. (Gift sample), Butylated Hydroxytoulene, Godrej ind. Ltd. (Gift sample), Butylated Hydroxyanisole, Godrej ind. Ltd. (Gift sample), distilled water. The cultures used for testing were, Propionibacterium acnes (MTCC) was purchased from the M.T.C.C, Institute of Microbial Technology, Chandigarh (India). Staphylococcus Epidermidis (MTCC) was purchased from the M.T.C.C, Institute of Microbial Technology, Chandigarh (India).

Preparation of Test Extract[3-5]

All plants were collected from Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha and authenticated by Dr. Mohan R. Agrawal, Sitabai Thite College of Pharmacy, Shirur. Voucher specimen was deposited. Extraction was carried out at room temperature under normal condition. Drugs were powered and passed through sieve no #10 and subjected to soxhlet extraction using petroleum ether and ethanol successively. Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethanol in a closed flask for 24 hours, shaked frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of ethanol, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105^0c and weigh. Percentage of ethanol-soluble extractive was calculated with reference to the air-dried drug.

Phytochemical Evaluation[6]

Plant is a biosynthetically prepared in laboratory not only for the primary metabolites such as carbohydrates, proteins and lipid that are utilized as food by man but also for secondary metabolites like glycosides, alkaloids, volatile oil, tannins etc. that exerts a physiological and therapeutic effects. The compounds that are responsible for medicinal property of drug are usually secondary metabolite. A systematic study of crude drug embraces through consideration of primary and secondary metabolites. Hence, plant material is subjected to preliminary phytochemical screening for detection of various Chemical constituents. Phytochemical evaluations were carried out for all the extracts as per standard procedure. (Brain & turner 1975, Evans 1996).

Formulation of Topical Herbal Cream[7,8]

The formulation of soy-phytosome topical cream consists of two stages:

1. Preparation of cream base (oily phase) and aqueous phase.
2. Formulation of Topical Herbal Cream.

Formulation Design

For preparation of topical herbal cream, it is proposed to use the following formulation composition in Table-01.

Table-01: Formulation composition of topical herbal cream(F019)

Sr. No	Ingredient	% w/w F017	Uses	Limits (%)
1	*Azadirachta indica*	1	Active	--
2	*Amba haldi*	1	Active	--
3	*Curcuma longa*	0.75	Active	--
4	White soft paraffin	10	Stiffening agent and emollient	15
5	Cetostearyl alcohol	6	Stiffening agent	12
7	Cetyl alcohol	4	Stiffening agent	12
8	Isopropyl Myristate	10	Penetration enhancer	10
9	Mineral Oil	5	Emollient	40
10	Sorbitan Monostearate	1.2	Emulsifier	8
11	Polysorbate 60	2.8	Emulsifier	5
12	Methyl Paraben	0.2	Preservative	0.3
13	Propyl Paraben	0.02	Preservative	0.08
14	Butylated Hydroxytoulene	0.1	Antioxidant	2
15	Butylated Hydroxyanisole	0.1	Antioxidant	0.05
16	Purified Water	q.s.	q.s.	q.s.

Procedure

According to above formulation composition all substances were weighed accurately. Then 10.0 g white soft paraffin, 6 gm cetostearyl alcohol, 4 g cetyl alcohol, 5 g mineral oil, 10 g Isopropyl Myristate, 0.1g Span 60 Butylated Hydroxytoulene and 0.1 g Butylated Hydroxyanisole (oily phase) were mixed in separate beaker and melted at water bath on temperature range of 65-70^{o}C, similarly remaining materials except plant extract were mixed in another beaker and heated on water bath at temperature range of 65-70^{0}C. Further molten mass of oily phase was added into other (aqueous) phase with continuous stirring was added and then cooled down to an emulsified cream. When

temperature reaches to 45-50^{0}C adds the extracts in purified water under stirring to dissolve it completely. Cool the bulk obtained up to 30°C -35°C under stirring. Fill the bulk obtained in proposed packing material.

Evaluation of topical herbal cream[7,8]

Various evaluation tests were performed on prepared formulations to evaluate various physicochemical and biological parameters, as follows:

1. Appearance
2. Determination of pH
3. Extrudability
4. Rheology (Viscosity)
5. Spreading Coefficient (Spread ability)
6. Diffusion studies
7. Skin irritancy test

Bacteriological Studies[9,10]

Preparation of petriplates: Petri dishes (Borosil, 4" diameter) and pipettes (1 ml) were sterilized by dry heat in a hot air oven at 160°C for 1 hour. The nutrient agar was sterilized by autoclaving at 121°C for 20 minutes at 15 lbs. pressure and used for the preparation of the plates.Agar cup plate method or Agar diffusion method was followed for the present investigations.

Method for Propionibacterium acnes

1. Propionibacterium acnes was incubated in brain heart infusion medium (BHI) with 1% glucose for 72 hr under anaerobic condition and adjusted to yield approximately 1.0 x 108 CFU/ml.
2. Aliquots of molten BHI with glucose agar were used as an agarbase.
3. Prepared inoculums was added to molten agar, mixed and poured over the surface of agar base and left to solidify.
4. Uniform-sized (6 mm diameter) filter-paper disks were impregnated with formulation and then placed on the surface of an agar plate that had been seeded with the organism to betested
5. These plates were then kept into McIntosh Fields anaerobic jar evacuated by pump and filled twice with nitrogengas.
6. This jar was incubated at 37°C for 72hours.
7. The zones of inhibition produced were recorded
8. The solvent blanks served ascontrols.

Method for Staphylococcus epidermidis

1. Staphylococcus Epidermidis was incubated in tryptic soy broth (TSB) for 24 hr at 37°C and adjusted to yield approximately 1.0 x 108 CFU/ml.
2. Aliquots of molten tryptic soy broth (TSB) were used as an agar base.
3. Prepared inoculums was added to molten agar, mixed and poured over the surface of agar base and left to solidity.
4. Uniform-sized (6 mm diameter) filter-paper disks were impregnated with formulation and then placed on the surface of an agar plate that had been seeded with the organism to be tested
5. These plates were then kept into McIntosh Fields anaerobic jar evacuated by pump and filled twice with nitrogen gas.
6. This plate was incubated at 37°C for 24 hours under aerobic condition.
7. The zones of inhibition produced were recorded.
8. The solvent blanks served as controls.

All disc diffusion tests were performed in three separate experiments and the antibacterial activity expressed as the mean of inhibition diameters (mm).

RESULT AND DISCUSSION

Table-02: Phytochemical Screening of ethanolic extracts of *Azadirachta indica, Amba Haldi and Curcuma Longa*

EL: Ethanolic extracts				
S. NO	**Plant Constituents**	**Azadirachta indica (Bark)**	**Amba Haldi**	**Curcuma Longa**
1	Alkaloids	-	-	+
2	Carbohydrates	+	-	+
3	Glycosides	+	-	-
4	Saponins	+	-	-
5	Phytosterol	+	-	-
6	Fats & Oil	-	-	-
7	Phenols	+	-	+
8	Tannins	+	-	-
9	Flavinoids	+	+	+
10	Proteins	-	+	-
11	Amino Acid	+	+	+

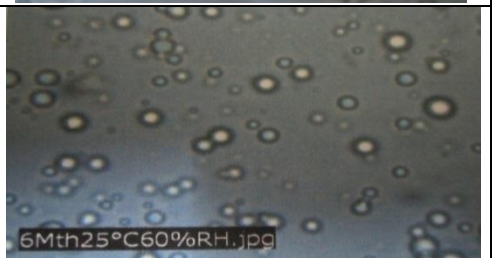
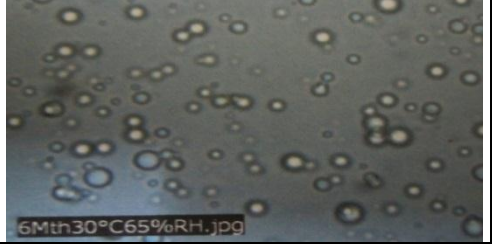
Table-03: Physical observation of Optimized Topical Herbal Formulation (Initial and 6th Month)

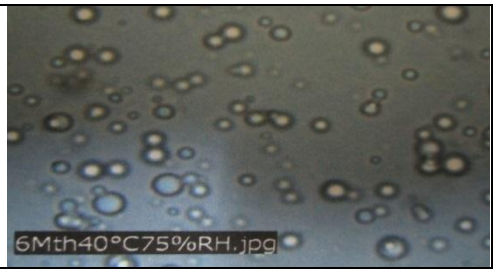
Sr. No.	Physical Parameters	Initial	Stability Conditions (6th Month)		
			25°C/60%RH	30°C/65%RH	40°C/75%RH
1	Appearance	Yellowish Cream	Yellowish Cream	Yellowish Cream	Yellowish Cream
2	Texture Analysis:				
a.	*Stiffness*	Smooth	Smooth	Smooth	Smooth
b.	*Grittiness*	Free from grittiness	Free from grittiness	Free from grittiness	Free from grittiness
c.	*Greasiness*	Free from Greasiness	Free from Greasiness	Free from Greasiness	Free from Greasiness
d.	*Tackiness*	Free from Tackiness	Free from Tackiness	Free from Tackiness	Free from Tackiness
e.	*Spreadability*	Easily spread	Easily spread	Easily spread	Easily spread
f.	*Stickiness*	Non sticky	Non sticky	Non sticky	Non sticky
g.	*Oiliness*	Non-oily	Non-oily	Non-oily	Non-oily
3	Emulsion type	O/W	O/W	O/W	O/W
4	Phase Separation	No phase separation	No phase separation	No phase separation	No phase separation
5	Ease of Application	Easily applied	Easily applied	Easily applied	Easily applied
6	Skin Irritation	No skin irritation	No skin irritation	No skin irritation	No skin irritation
7	pH	5.24	5.22	5.22	5.21
8	Viscosity (Poise)	3.164	3.112	3.122	3.090
9	Torque (%)	50.667	50.330	51.000	51.000
10	Weight of Cream Extruded (g)	12.509	12.633	12.782	12.734
11	% Extrudation	84.39	84.22	85.21	84.89
12	Grade	++	++	++	++

The optimized formulation of cream does not show much variation in physical parameters initially and after 6 month stability condition at 25°C/60%RH, 30°C/65%RH and 40°C/75% RH. Therefore, the formulation is stable.

Fig-01: Physical and microscopic evaluation of F019 at initial and 6 Month 25°C/60%RH, 30°C/65%RH, 40°C/75%RH

The microscopic evaluation of the sample is done initially and upto 6 month from stability testing e.g. 25°C/60%RH, 30°C/65%RH, 40°C/75 % RH. The results were obtained are as follows.

Stability Condition	Physical Evaluation Image	Microscopic images
Initial		
F019: 6M 25°C/60% RH		
F019: 6M 30°C/65% RH		

F019: 6M 40°C/75% RH		

It is observed from the above images that cream does not show any deformity in structure and shape initially and after 6 month stability condition also. Therefore the formulation is stable physically.

Table-04: Estimation of Zone of inhibition of optimized topical herbal formulation of cream (F019) against Propionibacterium Acnes

Time point	Stability condition	Zone of inhibition Propionibacterium Acnes(mm)			
		1	2	3	Mean* (± S.D)
Initial		20.40	19.80	20.70	20.30 ± 0.46
3 M	25°C/60%RH	19.80	20.20	19.20	19.73 ± 0.50
	30°C/65%RH	19.90	19.80	20.80	20.17 ± 0.81
	40°C/75%RH	20.20	20.30	19.70	20.07 ± 0.32
6 M	25°C/60%RH	19.50	20.50	20.10	20.03 ± 0.50
	30°C/65%RH	20.60	20.60	19.60	20.07 ± 0.58
	40°C/75%RH	20.30	20.20	20.90	20.47 ± 0.38

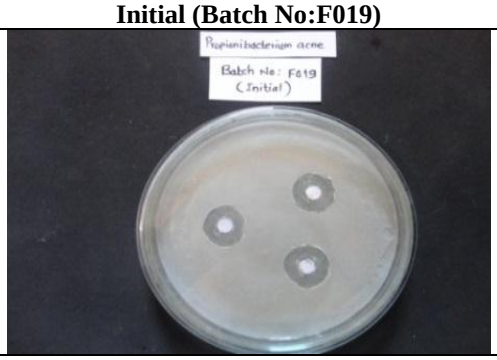
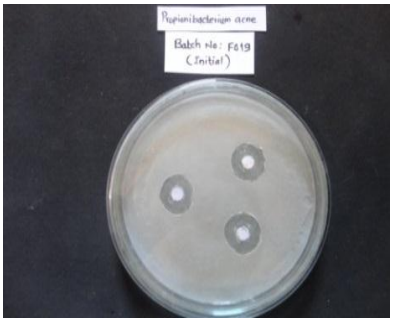
Where: * Mean of triplicate measurement

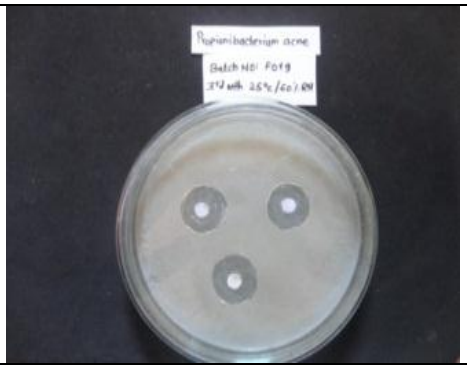
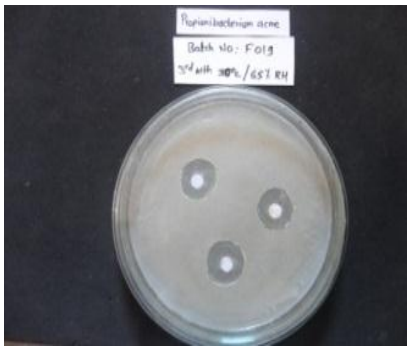
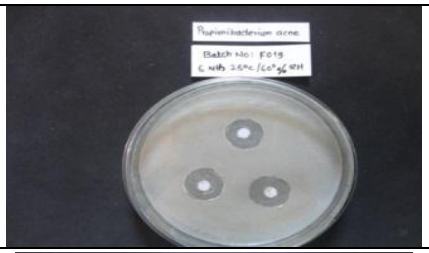
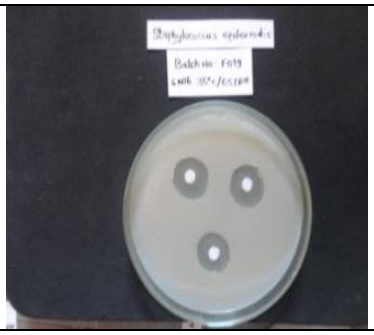
Table-05: Estimation of Zone of inhibition of optimized topical herbal formulation of cream (F019) against Staphylococcus Epidermidis

Time point	Stability condition	Zone of inhibition Staphylococcus Epidermidis (mm)			
		1	2	3	Mean* (± S.D)
Initial		21.30	22.10	22.10	21.83 ± 0.46
3 M	25°C/60%RH	20.80	22.20	21.60	21.53 ± 0.70
	30°C/65%RH	21.40	21.70	21.90	21.67 ± 0.25
	40°C/75%RH	22.10	21.80	20.80	21.57 ± 0.68
6 M	25°C/60%RH	21.20	22.40	21.30	21.63 ± 0.67
	30°C/65%RH	22.10	22.20	21.80	22.03 ± 0.21
	40°C/75%RH	20.90	22.10	21.40	21.74 ± 0.98

Where: * Mean of triplicate measurement.

Figure-02: Comparision of Zone of inhibition of optimized topical herbal formulation of cream (F019) and Clarina_16200463M (Marketed formulation) against *Staphylococcus Epidermidis* (initial and 6 Month stability) at different conditions

	Initial (Batch No:F019)	Clairna
Activity against Propionibacterium Acnes		

Activity against Staphylococcus Epidermidis			
Batch No:F019	**3rd M 25°C/60% RH**	**3rd M 30°C/65% RH**	**3rd M 40°C/75% RH**
Activity against Propionibacterium Acnes			
Activity against Staphylococcus Epidermidis			
Batch No:F019	**6th M 25°C/60% RH**	**6th M 30°C/65% RH**	**6th M 40°C/75% RH**
Activity against Propionibacterium Acnes			
Activity against Staphylococcus Epidermidis			

Where M=Month.

The optimized formulation of cream does not show much variation in zone of inhibition against *Propionibacterium Acnes* and *Staphylococcus Epidermidis* initially and after 3 month & 6 month stability condition at 25°C/60% RH, 30°C/65%RH and 40°C/75% RH. Therefore, the formulation is stable.

CONCLUSION

For topical application, alcoholic extracts of of *Azadirachta indica, Amba Haldi and Curcuma* Longa were incorporated into semisolid dosage form i.e. cream. It was a clear, transparent preparation with a uniform homogenous mass as no lump formation was observed. The viscosity and spreadibility was found to be as per the limits prescribed by standard pharmacopoeias. Thus this preparation by use of herbal drugs leads to less expensive over the commercial available product. Thus it can be conferred that there is a growing demand for herbal cosmetics in the world market and they are invaluable gift of nature. Herbal medications are considered safer than allopathic medicines as allopathic medicines are associated with the side effects.

REFRENCES

1. The Wealth of India", CSIR, New Delhi, 2003, volume IV, J-Q, PP4-5, 423-424.
2. Anil Kumar, Sanjula Baboota, SP Agarwal, Javed Ali, Alka Ahuja.,"Treatment of Acne With Special Emphasis on Herbal Remedies" Expert Review of Dermatology; 2008; 3(1): 111-122.
3. Mukhrjee Pulok K. Quality Control of Herbal Drugs 2002. Business Horizon, 5-34, 97, 98, 181-195, 248,249, 562-567.
4. Indian Pharmacopoeia 1996, Government of India, Ministry of Health and Family Welfare. Published by Controller, Publication and New Delhi: A-54, A-89.
5. British Pharmacopoeia 1998. Published on the recommendation of Medicine Commission pursuant to the Medicine Act 1968, Department of Health and social security Home and health Department, Ministry of Health and Social Services for Nothern Iseland. 2: A-139, A-140.
6. Roopashree T.S, Raman dang, Shobha Rani RH, Narendra C, "Antibacterial activity of antipsoriatic herb: cassia tora, momordica charantia and calendula officinalis" International journal of applied research in natural product, 2008; 1(3): 20-28.
7. Aniket, A. Kumari, P. Kumari, S. Saurabh, L. Khurana, K. S. Rathore, "Formulation and Evaluation of Topical Soy-Phytosome Cream" Indian Journal of Pharmacy and Pharmacology, April-June 2015; 2(2): 105-112.
8. Nidhi Chaurasiya, G.S. Chakraborthy, "Formulation and Evaluation of Herbal hydro gel from *Hibiscus Rosa-Sinensis"* Int J Curr Pharm Res 2014; 6(1): 14-16.
9. Choi et al., "In vitro antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, Propionibacterium acnes" J. Environ. Biol. 2011; 32: 313-318.
10. Mullika T. C., et al., "Antimicrobial effects of thai medicinal plants against acne inducing bacteria" Journal of Ethnopharmacology. 2011; 101: 330-333.